to test the hypothesis that subjects with TAO have a greater degree of tobacco dependence than do control subjects with coronary atherosclerosis (CAD).
Methods: Subjects with TAO (n = 218, confirmed by angiography, biopsy, or noninvasive arterial testing) or CAD (n=343, diagnosed by coronary angiography) were mailed a standardized questionnaire regarding tobacco use, to which 103 and 273 responded, respectively. Degree of tobacco dependence in each group was ascertained by several methods, including the Fagerström Test for Nicotine Dependence Questionnaire.
Results: The TAO group was younger at index date (year of first diagnosis for TAO patients, year of PTCA for CAD patients) (TAO 37.6±9.0 vs CAD 43.3±4.9 yrs, P<.0001), but the groups did not differ in age at first tobacco exposure (TAO 16.7±3.1 vs CAD 17.3±4.2 yrs, p=.67), current tobacco use at time of survey (TAO 54% vs CAD 46%, p=.17), or Fagerström score (TAO 4.7±2.3 vs CAD 5.1±2.3, p=.24). Kaplan Meier curves showed no significant difference in time to stopping tobacco use after first diagnosis (p=.798). TAO subjects smoked fewer cigarettes per day than CAD subjects (TAO 22.3±10.7 vs CAD 27.7±15.3 cigarettes/day, p=.003). However, among current smokers (n=153), the groups did not seem to differ in number of cigarettes/day (19.8±7.9 vs 22.5±11.1, p=.20). Individuals in the TAO group appeared to be more likely to have made a serious attempt to quit smoking than those in the CAD group (TAO 97%% vs CAD 90%, p=0.05).
Conclusions: In contrast to case reports of extreme tobacco dependence in the TAO population, the degree of tobacco dependence in subjects with TAO is similar to matched subjects with CAD.

POSTER SESSION

1200 Atherosclerosis

Tuesday, April 01, 2003, 3:00 p.m.-5:00 p.m.
McCormick Place, Hall A
Presentation Hour: 4:00 p.m.-5:00 p.m.

1200-134 Low Bone Mineral Density Predicts Significant Coronary Artery Disease at Cardiac Catheterization

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Background: Osteoporosis and coronary disease (CAD) share several risk factors (age, diabetes, hypertension [HTN], and smoking) and may be related. The purpose of this study is to investigate whether osteoporosis is predictive of significant CAD compared with traditional risk factors.
Methods: Dual energy x-rays absorptiometry (DEXA) scan and cardiac catheterization were performed on 209 patients (183 females, 26 males) between 1/2000 and 7/2002. DEXA scans were classified into 3 groups: normal, osteopenia, and osteoporosis based on World Health Organization criteria. Angiograms were classified as having significant CAD if ≥ 50% luminal stenosis was noted in a major coronary artery. Clinical variables (age, HTN, diabetes, smoking, family history of CAD, hyperlipidemia) were examined.
Results: 117 patients had significant CAD. Patients with and without CAD were similar clinically and demographically. Univariate predictors of CAD were osteoporosis, diabetes, hypertension, and family history of premature CAD (table). On multivariate analysis, odds ratio (OR) for CAD was highest for osteoporosis: OR=5.0 (95% confidence interval, 2.25-11.1 [P< 0.0001]).
Conclusion: The presence of osteoporosis predicts significant coronary stenosis with higher odds ratio than traditional risk factors. Our study is the first to report an increase in documented CAD in patients with osteoporosis.

Variable	Odds ratio	95% confidence interval	P- value
Osteoporosis	5	2.25 - 11.1	<0.0001
Hypertension	2.37	1.04 - 5.4	0.041
Family history of premature CAD	2.79	1.26 - 6.18	0.012
Diabetes mellitus	3.3	1.6 - 6.79	0.0012

1200-135 Rheumatoid Arthritis Is Not Associated With Myocardial Infarction After Controlling for Other Risk Factors

Jefferson T. Baer, Jesse A. Berlin, Daniel A. Albert, Stephen E. Kimmel, University of Pennsylvania, Philadelphia, PA

Background: Previous studies have suggested that patients with rheumatoid arthritis (RA) have an increased rate of cardiovascular death and myocardial infarction (MI) compared to the general population. However, the majority of these studies failed to control for known cardiac risk factors, some of which appear to be more common in patients with RA.
Methods: A case-control study of first MI, ages 40 through 75, was conducted among 36 hospitals in a 5-county area during a 3-year period. Cases were patients hospitalized with a first nonfatal MI, and controls were randomly selected from the same geographic area. Self-report of physician diagnosed RA was collected via telephone interviews, along with detailed information regarding cardiac risk factors and current medications using state-of-the-art methods. Multivariable logistic regression was used to adjust for age, sex, race, hormone replacement therapy, body mass index (BMI), cigarette smoking, insurance, exercise, income, education, history of coronary disease, family history, hypertension (HTN), diabetes mellitus (DM), hyperlipidemia, nonsteroidal anti-inflammatory drug use, aspirin use, disease-modifying anti-rheumatic drug use, and number of doctor visits.
Results: 1078 cases (4.74% with RA) and 4255 controls (3.62% with RA) were interviewed. There was an increased risk of myocardial infarction (MI) in patients with RA in the unadjusted model (OR 1.42, 95% CI: 1.02-1.97). However, after adjusting for confounding, there was no increased risk from RA (OR 0.86, 95% CI: 0.58-1.29). The variables responsible for confounding were age, HTN, DM, BMI, history of coronary disease, education and number of doctor visits. There were no subgroups at increased risk of MI from RA.
Conclusions: Previous studies showing an increased risk of cardiovascular death in patients with RA did not control for known cardiac risk factors. The current study showed an increased risk of MI in patients with RA that was solely explained by RA patients' greater prevalence of several risk factors. Physicians should aggressively screen for and modify common cardiac risk factors in patients with RA.

1200-136 Green Tea Derived Polyphenol Antioxidant, EGCG, Differentially Modulates Developing Compared to Mature Atherosclerotic Lesions in Apo E -/- Mice

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Background: Atherosclerosis starts at a young age, but therapy is generally begun when the disease is well established. Therapy such as green tea derived polyphenol antioxidant epigallocatechin gallate (EGCG) may be more effective when applied during the developing rather than established phase of atherosclerosis.
Hypothesis: Developing atherosclerotic lesions are differentially modulated by anti-oxidant therapy compared to mature atherosclerotic lesions.
Methods: Newly developing atherosclerotic lesions were induced by placing a carotid cuff in apoE (-/-) hypercholesterolemic mice at 28 weeks of age. EGCG (10 mg/kg, n=7) or PBS (n=7) was injected i.p. daily. Sections of cuffed arteries were stained for PCNA, H&E and oil-red-O. Aortic sinus plaques from the same animals were stained for collagen content and macrophage. Plasma cholesterol and antioxidant potential were also measured.
Results: Newly developed lipid-rich atheroma in the carotid artery were smaller in the EGCG group compared to the PBS group 21 days after cuffing. EGCG treatment reduced medial PCNA expression 3 days after cuffing (Table). Mature aortic sinus plaques were not affected by EGCG (Table). BW and cholesterol levels were comparable in both groups. EGCG level 1 hour after injection was 138 ± 44 ng/mL (n=5).
Conclusion: Our data suggest that antioxidant therapy inhibits the formation of developing, but not mature plaques. This observation implies timing of antioxidant therapy may be critical for antiatherogenic effect.

Group	Carotid PCNA (day 3)	Carotid plaque Size (mm^2)	Sinus Plaque Size (mm^2)	Sinus Plaque Macrophage (%)	Sinus Plaque Collagen (%)	Plasma antioxidant level#
EGCG	7.4±6.2* (37 sections)	0.014±0.008*	0.899±0.18	14.6±2.8	40.1±6.6	1.147 ± 0.579*
PBS	11.9±10.7 (25 sections)	0.031±0.013	0.851±0.18	17.2±4.6	42.5±3.2	0.262 ± 0.290

mean±std; *p < 0.05 vs. PBS group; #mM uric acid equivalents

1200-137 Downregulation of Microfilament Expression in Atherosclerotic Plaque Is Mediated by Oxidized Low-Density Lipoprotein

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The cellular microfilament system is a highly dynamic network comprised of multiple proteins that are essential for cell motility, contraction, cell-cell and cell-substrate interactions, signaling pathways, gene expression and cell differentiation. To examine the regulation of Vascular Smooth Muscle Cell (VSMC) microfilaments in atherosclerotic plaque development we performed immunostaining for α-, β-actin and vimentin in human femoral and carotid atherosclerotic plaques (n=20). There was a marked reduction in α-actin and vimentin expression in VSMC in advanced plaque. To identify stimuli that downregulate microfilament components in plaque, we incubated human VSMC with 0-100 ug/ml Oxidized LDL (OxLDL), an important proatherogenic molecule, or 0-100 ug/ml native LDL (nLDL) and measured cytoskeletal protein expression by western immunoblotting. OxLDL, but not nLDL, dose dependently reduced α-actin and vimentin expression (21 ± 5%, n=8, p<.05, and 52 ± 6%, n=19, p<0.0001, respectively, with 100 ug/ml OxLDL) and non-significantly increased β-actin expression. To determine intracellular signaling pathways mediating OxLDL effects on microfilaments we studied the effect of candidate proteins implicated in OxLDL signaling. OxLDL components are high-affinity ligands for the PPARγ nuclear receptor, but the effect of OxLDL was not blocked by the PPARγ inhibitor, PGF2 α, 200 nM, and the synthetic PPARγ ligand ciglitazone (0-10 uM) did not regulate vimentin or α-actin expression. Furthermore, inhibition of the p38 MAPK pathway (SB203580, 10 uM) did not modify OxLDL effects, indicating that the ability of OxLDL to regulate vimentin/α-actin was not mediated via a p38 MAPK dependent pathway. In conclusion:

1. There is a dramatic downregulation of α-actin and vimentin microfilaments in atherosclerotic plaque.
2. OxLDL markedly downregulates VSMC α-actin and vimentin expression via a PPARγ and p38 MAPK independent pathway. OxLDL regulation of VSMC cytoskeletal proteins may play a critical role in atherogenesis through alterations in cell motility, differentiation and growth.

1200-138 Intravascular Detection of Inflammed Atherosclerotic Plaques With a Novel Macrophage-Targeted Fluorescent Photodynamic Compound

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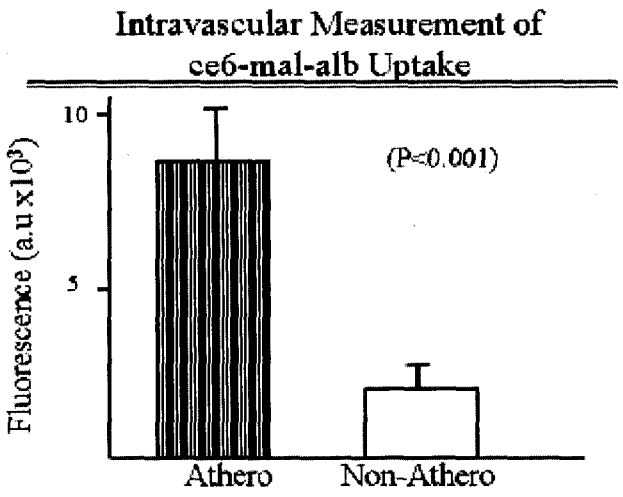
Background: Vulnerable plaques contain abundant macrophages. We developed a novel photodynamic agent, chlorin$_{e6}$ conjugated with maleylated albumin, (ce6-mal-alb) that concentrates in macrophage-rich plaques and has a high fluorescent yield. As such, we tested hypothesis that experimental atherosclerotic lesions (ATHERO) can be detected using ce6-mal-alb and an intravascular fluorescence spectroscopy catheter.
Methods: ATHERO were induced in New Zealand rabbits by infradiaphragmatic aortic balloon-injury followed by high cholesterol diet. At 10 weeks, ce6-mal-alb was administered to 7 atherosclerotic and 7 control animals. 24 hours later, aortic uptake of the ce6-mal-alb uptake was measured *in situ*, using an intravascular fluorescence spectroscopy catheter. Thereafter, the aortas were excised and dissolved in NaOH/SDS for spectrofluorimetric determination of ce6 content.
Results: Intravascular measurements of ce6-mal-alb fluorescence were higher in ATHERO vs. non-athero segments, (8.8±4.8 vs. 2.2±2.2 X10^{3}AU, P<0.001, figure). Further, ce6-mal-alb concentration was higher within the ATHERO aortas (5.2±3.2 vs. 1.9±1.2, ce6 fluorescence/gm tissue x 10^6, p=<0.01). **Conclusion**: Ce6-mal-alb can be employed for intravascular characterization of atherosclerotic plaques. Because this novel PDT compound is selectively toxic to macrophages when light-activated, this agent may be useful for both the detection and therapeutic modification of macrophage-rich plaques.



1200-139 Endogenous Free Radical Generating Sources Are Involved in Smoking-Mediated Dysfunction of Nitric Oxide Biosynthesis in Human Coronary Artery Endothelial Cells: An In Vitro Demonstration

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Background We have previously demonstrated that cigarette smoking alters NO biosynthesis by reducing NO availability and eNOS activity while increasing eNOS protein expression. Although oxidative stress has been proposed as the leading mechanism for these changes, the source of free radicals is unclear. Cigarette smoke contains high levels of free radicals. However, free radicals potentially arise from endogenous sources as well. This study investigated the role of endogenous free radical generating sources in smoking-related dysfunctional NO biosynthesis in human coronary artery endothelial cells (HCAECs).
Methods and Results Confluent (~85%) monolayers of HCAECs were incubated with serum from 6 nonsmokers and 8 smokers for 12 hours with or without the addition of either 100uM LNMMA, 30uM apocyanin (NADPH oxidase inhibitor), 100uM allopurinol (xanthine oxidase inhibitor) or 2uM rotenone [mitochondrial electron transport chain (METC) inhibitor]. Following incubation, NO levels and eNOS protein expression were measured from the same culture by standard techniques. HCAECs incubated with smokers' serum alone showed significantly lower NO level (0.02±0.01versus 0.13±0.02 uM/pg eNOS/mg total protein, P<0.007) and higher eNOS expression (P<0.005) compared to nonsmokers. In smokers, only allopurinol (0.04±0.01 uM/pg eNOS/mg total protein) or rotenone (0.06±0.01 uM/pg eNOS/mg total protein) treatment significantly P<0.05) improved NO availability. However, levels were still lower compared to nonsmokers (P<0.05). Interestingly, when smokers' serum was treated with combined rotenone, allopurinol and 20uM tetrahydrobiopterin (an eNOS cofactor) the NO level, eNOS expression and eNOS activity normalized to that of nonsmokers.
Conclusions To our knowledge this is the first demonstration that endogenous free radical generators such as xanthine oxidase, the METC and eNOS contribute to smoking-mediated dysfunction of NO biosynthesis.

1200-140 Synergistic Effect of Urotensin II With Serotonin on Vascular Smooth Muscle Cell Proliferation

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Background: Urotensin II (U-II), the most potent vasoconstrictor known to date, and serotonin (5-HT) have been recently shown to play an important role in pulmonary hypertension. However, little is known about the effect of U-II and its interaction with 5-HT on vascular smooth muscle cell (VSMC) proliferation. We assessed the interaction between U-II and 5-HT in inducing VSMC proliferation.
Methods: Growth-arrested rabbit VSMCs were incubated in serum-free medium with different concentrations of U-II and 5-HT. VSMC proliferation was examined by the increase in [3H]thymidine incorporation into cellular DNA and cell number.
Results: U-II or 5-HT induced [3H]thymidine incorporation in a concentration-dependent manner with a maximal effect at a concentration of 50 nanoM (161%) or 50 microM (205%), respectively. When added together, low concentrations of U-II (50 nanoM) and 5-HT (1 microM) interacted synergistically in inducing [3H]thymidine incorporation (382%). These effects on [3H]thymidine incorporation were paralleled by an increase in cell number. The G-protein inactivator GDP-beta-S (100 microM), protein kinase C (PKC) inhibitor Ro31-8220 (0.1 microM), c-Src tyrosine kinase inhibitor PP2 (1 microM), and mitogen-activated protein kinase (MAPK) kinase inhibitor PD098059 (10 microM) significantly inhibited the mitogenic effects of U-II and 5-HT and also their interaction in inducing [3H]thymidine incorporation.
Conclusion: Our results suggest that U-II and 5-HT may induce the synergistic interaction in inducing VSMC proliferation via a G-protein-coupled receptor/PKC/c-Src tyrosine kinase/MAPK pathway, thus contributing to the relatively rapid development of atherosclerosis in hypertensive vascular disease.

1200-141 Systemic Inflammation: Independent Predictor of Contrast Induced Nephropathy

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Background: Recent observations demonstrate that systemic inflammation may exert adverse influence throughout the vascular bed. This study was designed to determine whether there is a relationship between the presence and magnitude of systemic inflammation and contrast induced nephropathy in patients undergoing coronary angiography.
Methods: In 244 patients undergoing coronary angiography we analyzed the association between systemic markers of inflammation (high sensitivity C-reactive protein, CRP) and contrast induced nephropathy (CIN) defined as decrease in creatinine clearance (CC) of at least 25 cc/min. Univariate and multivariate analysis were performed.
Results: Mean age of the patients was 62±13 yrs and 61% we males. Baseline CC was 89 cc/min. 82% underwent angiography for acute coronary syndrome. 8% developed CIN following the angiography. 2% of the patients in the first tertile of CRP develped CIN compared to 12% in second and 13% in the third tertile of CRP, p=0.03. On multivariate analysis higher CRP tertile was an independent predictor of CIN (OR 2.8, p=0.02) along with use of intra-aortic balloon pump (OR=9.3, p=0.04) and baseline creatinine clearance (OR=1.04, p<0.0001).
Conclusion: These findings demonstrate a strong association between the presence and magnitude of systemic inflammation and development of contrast induced nephropathy.

1200-142 Stabilization of Plaque Size Accompanied by a Time Dependent Decrease in Activated Macrophage Content After Vascular Injury in the Apolipoprotein-E Null Mouse

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Background: Apolipoprotein-E knockout (ApoE$^{-/-}$) mouse models have been used to study atherosclerosis and responses to vascular injury. Wire injury of the carotid artery results in characteristic neointima formation 28 days after injury. The purpose of this study was to characterize plaque size and cellular content at 2 and 3 months after carotid injury to identify changes in lesion progression.
Methods: Sixteen female ApoE$^{-/-}$ mice were fed a Western diet for one week prior to wire denudation of the left common carotid artery and continued on the atherogenic diet for 4 weeks. After 4 weeks, they were fed a chow diet until sacrifice. Mice were euthanized at 2 months (n=8) and 3 months (n=8) after injury and fasting blood was taken for glucose and cholesterol measurements. Vessels were harvested and paraffin embedded for morphology and immunocytochemistry. The results were compared to 1 month historical controls.
Results: At 1 month, plaque size was 30,500 ± 5,300 μm^2 , macrophage content was 21± 5 %, and cholesterol level was 1170 ± 210 mg/dl. At 2 and 3 months, neointimal areas were not statistically different (35,177 ± 6,789μm^2 vs. 29,196 ± 7,312μm^2 respectively) and similar to the 1 month group. The presence of activated macrophages in the neointima was significantly higher at 2 months compared to 3 months (26.2 ± 5.7 % vs 4.2 ± 1.5 %, p = .002). There were no significant differences in mean glucose and cholesterol levels at 2 and 3 months (107 ± 7 vs. 108 ± 11 mg/dl and 427 ± 20 vs. 435 ± 35 mg/dl respectively).